The acceleration brought about by the addition of sugars may be attributed to a withdrawal of water by the sugar and the consequent increase in the amount of the "active system," as pointed out in our previous paper.

"Studies on Enzyme Action: The Effect of 'Poisons' on the Rate of Decomposition of Hydrogen Peroxide by Hæmase." By GEORGE SENTER, Ph.D., B.Sc. (Lond.). Communicated by Professor E. H. STARLING, F.R.S. Received June 2, 1904.

Schönbein* was the first to observe that most animal and vegetable juices have the property of decomposing hydrogen peroxide into water and oxygen, as well as the power of developing a blue colour in tincture of guaiacum containing a little hydrogen peroxide. Since these properties belonged to all the enzymes then known, *e.g.*, emulsin and diastase, Schönbein regarded them as characteristic of enzymes in general, and used them in his numerous investigations as tests for the presence of these bodies.

A good many years afterwards Jacobson,† working with impure emulsin and pancreatin, showed that the power of these enzymes to catalyse hydrogen peroxide could be destroyed without affecting seriously the specific ferment action. A year or two ago, Loew‡ suggested that the power of plant and animal juices to decompose hydrogen peroxide is due to an enzyme of very wide distribution, which he has named catalase. According to this view, Jacobson's impure emulsin contained some catalase, which is less resistant against heat than the emulsin, and can be rendered inactive without affecting seriously the other ferment.

Since Schönbein's time, the properties which blood possesses of decomposing hydrogen peroxide and of giving the guaiacum reaction have formed the subject of numerous investigations by Schmidt,§ Bergengrün,‖ Spitzer,¶ Schär,** Cotton,†† Ville and Moitessier,‡‡ and others, and this is not surprising when we consider the importance of the guaiacum test for the detection of small amounts of blood.

Spitzer§§ concluded that the guaiacum reaction and the catalysis

* 'Journ. f. prakt. Chemie,' vol. 89, p. 334 (1863).
† 'Zeit. f. physiol. Chemie,' vol. 16, p. 340 (1892).
‡ Loew, "Catalase," 'U.S. Dept. of Agriculture Report,' No. 68, 1901.
§ Pflüger's 'Archiv,' vol. 6, p. 413 (1872).
‖ 'Inaug. Dissertation,' Dorpat, 1888.
¶ Pflüger's 'Archiv,' vol. 67, p. 615 (1897).
** 'Zeit. für Biologie,' vol. 6, p. 467 (1870).
†† Cotton, 'Bull. Soc. Chim.,' vol. 25, p. 255 (1901).
‡‡ 'Bull. Soc. Chim.' [3], vol. 27, p. 1003 (1902).
§§ *Loc. cit.*

of hydrogen peroxide are due to the same substance, and this has been the generally accepted opinion* until very recently. Schmidt long ago suggested that the catalysis of hydrogen peroxide by blood is due to the hæmoglobin, and though this view had been combated by Kobert,† Schär,‡ and others, no very definite evidence had been brought forward on either side.

Last year the author§ succeeded in preparing from defibrinated ox blood a body which energetically decomposes hydrogen peroxide and does not give the guaiacum reaction. This body, which has so far been obtained only in solution, has been named hæmase.

In the former paper‖ it has been shown that hæmase rapidly loses its activity when heated in dilute aqueous solution to 60°; that in moderately dilute hydrogen peroxide solutions hæmase undergoes slow oxidation by the peroxide simultaneously with the catalysis of the latter, but that in dilute (n/100) peroxide solutions the latter is split up without the catalysor being affected. It has also been shown that in dilute solution the velocity of the decomposition of the peroxide is, within fairly wide limits, proportional to the hæmase concentration and to the concentration of the hydrogen peroxide. Preliminary investigation of the effect of other substances on the reaction showed that it is greatly retarded by very small amounts of acids and alkalis and to a much smaller extent by sodium chloride.

As regards the nature of the catalysor in question there seems good reason to regard it as belonging to that rather indefinite class of substances known as enzymes. In the present state of our knowledge no good definition of an enzyme can be given. We may regard them as substances formed by living cells which can be separated from the latter without losing their activity, and under whose influence certain chemical changes are brought about in a catalytic manner. They are soluble in water, precipitated by alcohol, destroyed by heating in aqueous solution to 60° or 70°, and their activity is often greatly influenced by small traces of foreign bodies. In all respects hæmase conforms to the above definition.

Since hæmase (in aqueous solution at 0°) is exceedingly stable and the velocity measurements are so accurately reproducible on different occasions (remarkably so for an enzyme action), the investigation has been continued with the hope of throwing some light on the nature of the enzyme, and the present communication contains an account of the influence of various substances on the rate of reaction.

* *Vide* Oppenheimer, 'Ferments,' p. 45.

† 'Naturforscherversammlung Aachen,' 1900, quoted by Bredig, 'Anorganische Fermente,' Leipzig, 1901, p. 88.

‡ 'Zeit. fur Biologie,' vol. 19, p. 330 (1899).

§ 'Zeit. für physik. Chemie,' vol. 44, p. 257 (1903).

‖ Senter, *loc. cit.*

Method of Measuring the Reaction Velocity.

All the experiments mentioned in the present communication were carried out in a thermostat at a temperature of 10° (constant to $\frac{1}{10}$°). Ninety cubic centimetres of the very dilute enzyme solution was placed in each bottle, 10 c.c. of a solution of the substance whose action was to be investigated (respectively 10 c.c. of water in the control experiments) added; after the lapse of a certain time (period of incubation) 100 c.c. of approximately $n/50$ hydrogen peroxide (also at 10°) added, and the mixture shaken. From time to time 25 c.c. of the mixture were pipetted out, run into sulphuric acid, which stops the action, and titrated with $m/500$ permanganate.

It may be mentioned that the enzyme solution was so active, compared with the amount of organic matter present, that no appreciable error (less than $\frac{1}{10}$ c.c.) was caused through the reduction of $KMnO_4$ by organic matter. The hydrogen peroxide used was obtained from Merck and was quite pure.

The enzyme solution was prepared according to the method given in my former paper,* and being kept in an ice-box retained its activity without appreciable change for several days.

Nearly all the substances used in the experiments were obtained from Kahlbaum, the others were the purest obtainable in London.

The water used for dilution was distilled water freed from carbon dioxide by the passage of a current of pure air through it for some time.

Effect of Acids on the Reaction Velocity.

In order to show clearly the nature of the results obtained, I give here (p. 204) full details of measurements on the effect of HCl, H_2SO_4, and HNO_3 on the reaction-velocity. In order to economise space, only the "constants" of subsequent experiments will be given.

The value of the constant in the control experiment was 0·0250. The numbers under $C_{H_2O_2}$ give the concentration of the H_2O_2 at the time indicated, expressed in cubic centimetres of 1/500 molar $KMnO_4$. The constants in the third column are calculated on the assumption that the reaction-velocity is proportional to the H_2O_2 concentration, that is, that $-\frac{dC_{H_2O_2}}{dt} = K_1 C_{H_2O_2}$ where $C_{H_2O_2}$ is the concentration at the time t. Integrating, we obtain $0{\cdot}4343K_1 = \frac{1}{t_2 - t_1} \log \frac{C_1}{C_2}$, where C_1 and C_2 are two successive measurements, and $t_2 - t_1$ the interval of time between them.

The effect of hydrochloric acid, sulphuric acid, nitric acid, benzoic acid, succinic acid, and acetic acid, on the rate of reaction has been

* *Loc. cit.*

Table I.—Initial Concentration of H_2O_2 in the Reaction Mixture approx. $n/200$.

t (min.).	$C_{H_2O_2}$.	$0{\cdot}4343K_1$.	t (min).	$C_{H_2O_2}$.	$0{\cdot}4343K_1$.
HCl $n/40{,}000$.			HCl $n/100{,}000$.		
0	22·7	—	0	22·7	—
8·5	19·8	0·0070	8	17·3	0·0147
23·5	15·5	0·0071	23	10·3	0·0150
39·5	11·8	0·0074	38	6·2	0·0150
68·5	7·4	0·0070	53	3·7	0·0150
HNO_3 $n/40{,}000$.			HNO_3 $n/100{,}000$.		
0	22·7	—	0	22·7	—
105	21·2	0·0003	41	19·1	0·0018
225	19·2	0·0003	71	16·7	0·0019
			130	12·6	0·0020
			250	7·0	0·0021
			405	3·1	0·0022
H_2SO_4 $n/40{,}000$.			H_2SO_4 $n/100{,}000$.		
0	22·7	—	0	22·7	—
10	19·5	0·0066	9	16·7	0·0148
25	15·1	0·0074	24	9·9	0·0151
40	11·8	0·0071	39	5·9	0·0150
70	7·1	0·0073	54	3·5	0·0150
130	2·8	0·0068	—	—	—

investigated. Results for the first three acids have been given above; the following are the results obtained for the other three, with the numbers for HCl, obtained at the same time, for the sake of comparison:—

Table II.

Concentration of acid.	Concentration of H-ions.	Constants.
Acid, hydrochloric, $n/10{,}000$	$n/10{,}000$	0·0015
,, ,, $n/20{,}000$	$n/20{,}000$	0·0034
,, benzoic, $n/4{,}000$	$n/11{,}000$	0·0019
,, ,, $n/10{,}000$.....	$n/19{,}000$	0·0033
,, ,, $n/25{,}000$.....	$n/37{,}000$	0·0078
,, succinic, $n/10{,}000$.....	$n/18{,}200$	0·0029
,, ,, $n/25{,}000$.....	$n/36{,}000$	0·0070
,, acetic, $n/1{,}333$	$n/10{,}000$	0·0041
,, ,, $n/2{,}666$	$n/13{,}000$	0·0049
Control experiment	0	0·0300

From the tables, it will be seen that, while HCl and H_2SO_4, which are completely dissociated under the conditions of experiment, give

exactly the same retardation, benzoic, succinic, and acetic acids, which are only partly dissociated, have considerably less effect in equivalent concentration; when, however, the concentration in hydrogen ions is calculated (see second column), HCl, H_2SO_4, benzoic acid and succinic acid have exactly the same retarding effect, that is, the retardation is proportional to the hydrogen ion concentration. Nitric acid is more, and acetic acid less, poisonous than would be expected from the hydrogen ions present, and it is natural, in these two cases, to suggest that the negative ions exert an influence. This has been shown to be the case, as will be mentioned more fully later. While the Cl and SO_4 ions exert comparatively little influence, potassium nitrate slows down the action to about the same extent as nitric acid itself. On the other hand, sodium acetate has more accelerating influence on the reaction than any other substance that has been tried.

I may here mention that, although from considerations of space only one series of results has been given, three series of measurements have been carried out at different times with different enzyme preparations, and exactly corresponding results obtained, so that the figures quoted may be accepted as reliable.

Another interesting point about the behaviour of acids is that the time during which the acid remains in contact with the enzyme before the H_2O_2 is added has no influence on the result; the equilibrium is attained within 5 minutes, and remains unaltered at the end of 2 or 3 hours. The change is not a permanent one, since if the acid, after an incubation period of 2 hours, be neutralised before the addition of the H_2O_2, the action proceeds with its original velocity.

Kahlenberg* and his assistants have made a systematic investigation of the toxic action of acids on small plants and on fishes, with the object of finding whether the electrolytic dissociation theory is capable of explaining the results. They find that the toxic effect is in the first instance proportional to the hydrogen ion concentration, though there are often secondary effects due to the other ions, and in all probability to undissociated molecules. Similar results have been obtained with seedlings by Cameron and Breazeale.†

Fernbach‡ and others have made systematic investigations of the effect of acids on enzyme actions. Fernbach gives a table containing the amounts of different acids just sufficient to inhibit the action of invertase on sugar, and from his numbers, and the dissociation constants of the respective acids given by Ostwald, I have calculated the hydrogen ion concentrations of the solutions in question; the results are given below :—

* Kahlenberg and Austin, 'Journ. phys. Chem.,' vol. 4, p. 553 (1900); Kahlenberg and Mehl, *loc. cit.*, vol. 5, p. 113 (1901).

† 'Journ. phys. Chem.,' vol. 8, p. 1 (1904).

‡ Thèse, Paris, 1890, quoted by Effront, 'Enzymes,' English Edit., 1902, p. 69, *et seq.*

Table III.

Acids.	Inhibiting quantity.	Hydrogen ion concentration.
Sulphuric acid.....	1/245 normal solution.	1/245 normal.
Tartaric „	1/75 „	1/280 „
Oxalic „	1/630 „	1/1000 „
Succinic „	1/30 „	1/700 „
Lactic „	1/9 „	1/265 „
Acetic „	5/6 „	1/266 „

It is clear that in this case, also, the retarding effect is, in the first instance, proportional to the chemical activity of the acid, though oxalic and succinic acids have a greater toxic action than would be expected.

Effect of Alkali upon the Reaction.

The influence of sodium hydroxide upon the reaction has been determined. It has been found that the velocity is not very appreciably influenced by solutions weaker than 1/2000 normal, but that in stronger solutions a retardation of the velocity is brought about. The retardation, unlike that caused by acids, depends upon the period of incubation, but the change is not a permanent one, since on neutralisation the original velocity is regained. Details are given in my former paper.*

Effect of Neutral Salts.

Numerous investigations of the influence of neutral salts on enzyme action have been carried out, but little of a general character can be deduced from the results. Dilute solutions have often a slightly accelerating action, which passes into a retardation in more concentrated solutions.

I have investigated the influence of halogens of the alkalis and alkaline earths, of the alkali sulphates, and of sodium acetate and carbonate. Only the halogen compounds exert a considerable retardation; the effects due to the others seem to be more of a secondary nature.

Some typical results are given below :—

Table IV.

	Constants.		Constants.
Lithium chloride, *m*/400	0·0059	Ammonium bromide, *m*/2000..	0·0136
Sodium chloride, *m*/400	0·0055	Barium chloride, *m*/200	0·0052
„ „ *m*/800	0·0093	„ „ *m*/400	0·0080
Potassium chloride, *m*/400 ..	0·0057	Strontium chloride, *m*/200....	0·0056
„ „ *m*/800 ..	0·0095	„ „ *m*/400....	0·0090
Ammonium chloride, *m*/400..	0·0058	Calcium chloride, *m*/200.....	0·0051
„ „ *m*/800..	0·0094	„ „ *m*/400.....	0·0084
Potassium bromide, *m*/800 ..	0·0075	Sodium fluoride, *m*/400	0·0170
„ „ *m*/2000..	0·0128	„ „ *m*/800	0·0246
Ammonium bromide, *m*/800..	0·0074	Control experiment	0·0280

* Senter, *loc. cit.*, p. 301.

Owing to the direct oxidising action of H_2O_2 upon the iodides, it has been found impossible to complete the series by making measurements with corresponding solutions of these compounds. This point will be again referred to under the experiments dealing with iodine.

From the results given above it is evident that the retarding effect due to the halogen compounds is due to the halogen ion alone, since it is quite independent of the other ion present. It may be mentioned in this connection that according to Bredig* the catalysis of hydrogen peroxide by colloidal platinum is retarded by KCl and NH_4Cl, though not appreciably so by other neutral salts.

The results with sodium fluoride are particularly interesting, since it has long been regarded as a poison for micro-organisms, but without effect on enzymes.† According to Pavy,‡ however, it has a slightly deleterious action on enzymes, and this is quite borne out by the results given above.

Alkali Sulphates.—Experiments were carried out with potassium, sodium, and ammonium sulphates, in concentrations varying from *m*/50 to *m*/400. The results are as follows :—

Table V.

Salt used.	Constants.	Salt used.	Constants.
K_2SO_4, *m*/50	0·0265	Na_2SO_4, *m*/50	0·0360
,, *m*/200	0·0235	,, *m*/200	0·0308
,, *m*/400	0·0245	,, *m*/400	0·0288
$(NH_4)_2SO_4$, *m*/50	0·0280	Control experiment; no salt present	0·0288
,, *m*/200	0·0250		
,, *m*/400	0·0260		

From the results given it is evident that, while K_2SO_4 and $(NH_4)_2SO_4$ exert a slight retarding action, Na_2SO_4 in *m*/50 solution has a considerable accelerating effect, which practically disappears in *m*/400 solution. As having a possible connection with this result it may be recalled that, of the three sulphates, only the sodium salt crystallises with water (10 molecules).

Sodium Acetate and Sodium Carbonate.—The results are as follows :—

Table VI.

Salt used.	Constants.	Salt used.	Constants.
Sodium acetate, *m*/100	0·0272	Sodium acetate, *m*/1000	0·0345
,, ,, *m*/200	0·0270	,, ,, *m*/2000	0·0340
,, ,, *m*/400	0·0250	Control experiment	0·0280
Control experiment	0·0180		

Salt used.	Constants.
Sodium carbonate, *m*/40	0·0070
,, ,, *m*/80	0·0135
,, ,, *m*/200	0·0194
Control experiment	0·0180

* Bredig, 'Anorg. Fermente,' p. 84.

† Oppenheimer, *loc. cit.*, p. 41.

‡ Pavy, 'Journ. of Physiology,' vol. 22, p. 391 (1898).

Quite an appreciable acceleration is caused by sodium acetate, and its amount seems, curiously enough, to be almost independent of the salt concentration. It has been found that neither sodium acetate nor sodium sulphate appreciably catalyse hydrogen peroxide in the concentrations employed.

Effect of Alkali Salts with Oxidising Properties.

In my former paper,* experiments with $KClO_3$ and KNO_3 are described, and it was shown that these salts at 0°, even in 1/80,000 molar solution, exert a powerful depressing effect on the action, the constants at the same time decreasing as if the enzyme were being gradually oxidised. There seems, however, to be a depressing effect independent of the oxidation since, with the weaker solutions, there is a considerable decrease in the velocity, though the constants do not appreciably decrease during the action. This is also evident from the experiment with nitric acid quoted on p. 204.

Experiments have now been carried out with potassium perchlorate and potassium persulphate, an experiment with $KClO_3$ being made at the same time for the sake of comparison.

Table VII.

Salt used.	Constants.	Salt used.	Constants.
$KClO_4$, *m*/50,000	0·0039	KSO_4, *m*/200.....	0·0076
,, *m*/100,000	0·0070	,, *m*/400	0·0137
,, *m*/250,000	0·0133	Control experiment	0·0300
$KClO_3$, *m*/1,000,000..	0·0054—0·0024		

It is interesting to note that the constants do not decrease during the action with potassium perchlorate and potassium persulphate, so that the enzyme is not being appreciably oxidised; there is, on the other hand, a marked decrease with potassium chlorate, from 0·0054—0·0024 in the course of 3 hours.

The results with potassium chlorate are interesting since this salt is a blood-poison, owing to its power of converting hæmoglobin into methæmoglobin. According to preliminary experiments by Professors Kobert and Schär,† potassium chlorate does not greatly retard the catalysis of hydrogen peroxide by blood; that this view is erroneous is evident from the results here given.

Potassium chlorate is not very poisonous towards the lower organisms.‡

* Senter, *loc. cit.*, p. 304.

† Quoted by Bredig, *loc. cit.*, pp. 81 and 85

‡ Loew, 'Die Giftwirkungen,' p. 17.

Effect of some Reducing Agents on the Reaction.

Measurements have been made with phenylhydrazine acetate, hydroxylamine hydrochloride, sulphuretted hydrogen, and formaldehyde, the results with the two former being interesting from their known property of combining with aldehyde and ketone groups.

Sulphuretted Hydrogen.—This body proved to be quite remarkably poisonous to the action, a solution containing 1/2,500,000 gramme-mol. reducing the rate to a half. The poisonous effect also depends greatly upon the period of incubation, the longer the incubation the greater the retardation. The constants also increase considerably during the action; this phenomenon is very likely due to the gradual oxidation of the H_2S by the H_2O_2.

The numerical results are appended:—

Table VIII.

Substance used and concentration.	Time of incubation.	Constants.
Sulphuretted hydrogen, *m*/500,000	10 mins.	0·0062—0·0085
,, ,, *m*/500,000	90 ,,	0·0018—0·0075
,, ,, *m*/1,000,000	10 ,,	0·0073—0·0080
,, ,, *m*/1,000,000	90 ,,	0·0025—0·0080
,, ,, *m*/2,500,000	10 ,,	0·0076—0·0100
,, ,, *m*/2,500,000	90 ,,	0·0054—0·0098
Without sulphuretted hydrogen	—	0·0190

It was observed by Faraday that platinum no longer brought about combination in detonating gas containing a little H_2S, and Bredig has shown that this gas is remarkably poisonous to the catalysis of H_2O_2 by colloidal platinum.*

Sulphuretted hydrogen has a slightly poisonous action on the lower organisms, but is a very energetic blood-poison, probably acting on the hæmoglobin, which it changes into a compound containing sulphur.†

Hydroxylamine.—This body has a considerably retarding effect, the constants decreasing slightly during the action.

Table IX.

Salt used.	Constants.
Hydroxylamine hydrochloride, *n*/20,000 ...	0·0008—0·0005
,, ,, *n*/40,000 ...	0·0047—0·0041
Without hydroxylamine	0·0180

Part of the hydrochloric acid remains uncombined and exerts a retarding action, but this is very small in so dilute a solution, and nearly the whole retardation is due to the hydroxylamine.

* Bredig, *loc. cit.*, p. 65.

† Loew, *loc. cit.*, p. 56.

Phenylhydrazine acetate does not retard the action quite so strongly as hydroxylamine; the constants decrease considerably during the action:—

Table X.

Substance used.	Constants.
Phenylhydrazine acetate, *m*/5,000.....	0·0040—0·0010
,, ,, *m*/10,000	0·0061—0·0025
,, ,, *m*/20,000	0·0093—0·0041

As in the former case, the free acid present retards the action, but only to a small extent.

Both hydroxylamine and phenylhydrazine are exceedingly poisonous to the lower organisms, much less so to the higher animals.* They both retard the catalysis of hydrogen peroxide by platinum to a considerable extent.† Very few observations of their effect on enzymes have been made.

Formaldehyde.—As is well known, this substance is very poisonous for the lower organisms, and is now largely used as an antiseptic.

According to Effront,‡ very minute amounts slow down the hydrolysis of starch by diastase, on the other hand, it has little influence on the activity of rennet.§ From the results given it is clear that formaldehyde only slightly affects the activity of hæmase:—

Table XI.

	Constants.
Formaldehyde, *m*/1000	0·0300
,, *m*/2000	0·0337
Without formaldehyde	0·0372

It may be mentioned that hydrogen peroxide does not appreciably oxidise formaldehyde in the dilution used in these experiments.

Effect of Mercuric Salts on the Reaction.

Experiments have been carried out with mercuric chloride, bromide, and cyanide. The two former have an exceedingly toxic effect, while the latter has very little action:—

Table XII.

Salt used.	Constants.	Salt used.	Constants.
$HgCl_2$, *m*/250,000...	0·0020—0·0004	$HgBr_2$, *m*/80,000 ...	0·0040—0·0009
,, *m*/500,000...	0·0033—0·0006	,, *m*/200,000 ..	0·0078—0·0032
,, *m*/1,000,000	0·0052—0·0018	$Hg(CN)_2$, *m*/400....	0·0154
,, *m*/2,000,000	0·0098—0·0054	,, *m*/800....	0·0213
$HgBr_2$, *m*/40,000....	0·0025—0·0013		
Control experiment without mercuric salt			0·0250

* Loew, *loc. cit.*, pp. 39, *et seq.*

† Bredig, *loc. cit.*, p. 76.

‡ Effront, 'Enzymes,' p. 117.

§ Oppenheimer, *loc. cit.*, p. 114.

Mercuric salts are energetic poisons for both higher and lower organisms.* Paul and Krönig† have shown that the poisonous action on bacteria diminishes from the chloride through the bromide to the cyanide, and it is known that the electrolytic dissociation decreases in the same order.

Mercuric chloride in the amount of one-millionth paralyses the action of diastase on starch‡ and, according to Bredig,§ is a very active poison for the platinum catalysis of hydrogen peroxide.

From the quantitative measurements given above it is clear that mercuric chloride is about five times as toxic as mercuric bromide towards the hæmase catalysis,‖ and at least 20,000 times as toxic as mercuric cyanide.

Effect of some other Poisons on the Reaction.

Carbon monoxide.—As is well known, carbon monoxide is very poisonous towards the higher animals on account of its property of forming a stable compound with the hæmoglobin of the blood. It was therefore a matter of interest to investigate its effect on the enzyme-catalysis of hydrogen peroxide, since the enzyme used is got from blood.

In order to get as much of the gas as possible dissolved, measurements were carried out at 0°; the gas was passed through a dilute solution of the enzyme in a bottle for 4 minutes, then, without stopping the current, the hydrogen peroxide was added and the stopper inserted, the bottle being thus filled with an atmosphere of the gas.

It was found that the gas exerted no appreciable poisoning effect; the constant in the presence of CO was 0·0085, and in its absence 0·0090, an agreement with the limit of experimental error.

Carbon monoxide is not poisonous towards the germination of seeds,¶ nor towards bacteria;¶ according to Buchner,** it does not affect the fermentation of sugar by zymase. It retards the catalysis of hydrogen peroxide by colloidal platinum.††

Iodine.—Iodine, as well as bromine and chlorine, are poisonous for all living matter,‡‡ and Bredig,§§ in his interesting experiments on the

* Loew, 'Die Giftwirkungen,' p. 35.

† 'Zeit. f. physik. Chemie,' vol. 21, p. 414 (1896).

‡ Effront, *loc. cit.*, p. 116.

§ Bredig, *loc. cit.*, p. 81.

‖ Up to the present the extent to which solutions of mercuric bromide are hydrolysed is not known, so that the relative ion concentrations of these solutions cannot be calculated, *vide* Luther, 'Zeit. phys. Chem.,' vol. 47, p. 107 (1904).

¶ Loew, *loc. cit.*, p. 103.

** See Ikeda, 'Zeit. physik. Chemie,' vol. 37, p. 26 (1901).

†† Bredig, *loc. cit.*, p. 78.

‡‡ Loew, *loc. cit.*, p. 16.

§§ Bredig, *loc. cit.*, p. 74

platinum catalysis of hydrogen peroxide, found that the first-mentioned substance, even in a dilution of 1 gramme-mol. in 7,000,000 litres, reduces the rate of reaction to half its original value.

I have used in my experiments iodine dissolved in water and also in aqueous solution of potassium iodide. The results are :—

Table XIII.

Substance used.	Constants.
Iodine dissolved in water, *m*/4000	0·0116—0·0160
„ „ „ *m*/8000	0·0168—0·0190
Iodine, *m*/40,000, potassium iodide, *m*/10,000 ...	0·0070
Control experiment without iodine	0·0185

The interesting result is thus obtained that an aqueous solution of iodine exerts only a very slight poisonous action on the enzyme, while a solution in potassium iodide, which contains the iodine in the form of I_3 ions, is distinctly poisonous, though very far behind what Bredig found for the platinum catalysis. Experiments carried out with potassium iodide in 1/10,000 molar solution show that the poisonous effect does not depend upon its presence, since the action was, if anything, accelerated under these conditions. It was observed, however, that the colour of the iodine solution partly disappeared on treatment with H_2O_2, so that some chemical change had evidently taken place.

Arsenious Oxide.—This body is a deadly poison for higher and lower organisms,* but has been found by various investigators to have only a slightly poisonous effect upon enzymes.† This is fully confirmed by the results here given :—

Table XIV.

Substance used.	Constants.
As_2O_3, *m*/2000.....	0·0224
„ *m*/4000.....	0·0213
Without As_2O_3	0·0214

From the total number of cubic centimetres of permanganate used, the amount used up by the As_2O_3 was subtracted.

The oxidation of the As_2O_3 by hydrogen peroxide is very slow in the dilution here employed, and it was found that this oxidation is not in the least accelerated by the simultaneous decomposition of the hydrogen peroxide.

Discussion of the Results.

The Mechanism of the Catalysis by Hæmase.—Before discussing generally the results obtained with poisons, the mechanism of the

* Loew, *loc. cit.*, p. 19.

† Kobert, *loc. cit.*, p. 153, Buchner, 'Berichte,' vol. 31, p. 2675.

catalysis of hydrogen peroxide by hæmase may be briefly considered. In this connection it must, in the first place, be taken into consideration that we are dealing with a heterogeneous reaction, since the hæmase is, in all probability, present in the solution in a colloidal state. According to our present views, such solutions form a two-phase system, the colloid being suspended in the liquid in a very fine state of division.

It has recently been insisted upon, more particularly by Nernst,* that we are not entitled to apply the equations governing reaction velocity in homogeneous systems to heterogeneous systems.

According to Nernst there is always equilibrium at the boundary surface between two phases, and the changes which take time are (1) chemical actions in the two phases, and (2) diffusion of substances to and from the boundary. Examples of the first type, in which the reaction velocity in one of the two phases is slow compared with the rate of diffusion, are given in the paper quoted; reactions of the second type, in which the velocity of diffusion determines the rate of action, have been experimentally investigated by Brunner.†

It may be taken as proved that the rate of solution of marble in acids is conditioned by a diffusion process, and the same is true of the rate of combination of hydrogen and oxygen in contact with a platinum surface. One criterion for the dependence of a reaction velocity upon diffusion is the effect produced by stirring the solution—this shortens the diffusion path, and thus increases the speed of reaction.

Nernst‡ is also of opinion that the same explanation holds for the decomposition of hydrogen peroxide by colloidal platinum—that the actual decomposition is very rapid compared with the diffusion of the peroxide to the surface of the catalysor.

We will now inquire whether the catalysis of hydrogen peroxide by hæmase can be represented in a similar way. Since the particles of a colloidal solution are in a continual state of motion, we may assume that stirring would not appreciably affect the reaction velocity. We may imagine that each particle of colloid has adhering to it a layer of liquid; that the rate of decomposition of the peroxide by the enzyme is very great, and that the concentration outside the adhering layer is kept constant by the motion of the colloidal particles. The rate of decomposition of the peroxide would then be determined by the rate of its diffusion through the adhering film, and, since the rate of diffusion is proportional to the difference of concentration on the two sides of the film, a simple explanation would be given of the observed fact that the reaction velocity is proportional to the concentration of the peroxide. Some support is given to this view by the fact that the

* Nernst, 'Zeit. physik. Chemie,' vol. 47, p. 52 (1904).

† Brunner, 'Zeit. physik. Chemie,' vol. 47, p. 56 (1904).

‡ *Loc. cit.*

temperature coefficient of the hæmase catalysis for 10° is 1·5, which agrees with that found by Brunner for the velocity of solution of benzoic acid in water, but is much smaller than the average for reactions in a homogeneous system.*

I have previously shown that while the reaction velocity in very dilute solutions is proportional to the hydrogen-peroxide concentration, in stronger solutions deviations occur,† which can be summed up by saying that the reaction proceeds more rapidly in the relatively more dilute peroxide solutions. On the diffusion hypothesis this can be simply explained on the assumption that the diffusion process is more disturbed in strong solutions by the oxygen given off from the surface of the particles.

While the above hypothesis affords a simple explanation of all the facts in the hæmase catalysis, it by no means follows that it can be extended to enzyme actions in general. The assumption made is that the reaction at the surface is much quicker than the diffusion changes concerned, and, while this is so in the dissolving of magnesia by acids, and, in all probability, in the catalysis of hydrogen peroxide by hæmase and by platinum, it may not be so in reactions between enzymes and more complicated substances.‡

As regards the catalysis itself, it may be said with certainty that it is not due to the large surface area of the colloid particles acting mechanically on the peroxide, since many colloidal solutions have no catalytic effect whatever. It may be due to the formation of a chemical compound between the peroxide and the enzyme, which, being unstable, breaks down into water, oxygen, and the enzyme, or the compound may enter into double decomposition with another molecule of the peroxide.§ Two observations which I have made may contribute something to the elucidation of these changes. As mentioned on p. 212 oxidations are not accelerated during the catalysis, so that

* van't Hoff, 'Vorlesungen,' vol. 1, p. 225.

† Senter, *loc. cit.*, p. 286.

‡ Since this paper was written my attention has been drawn to a paper by Herzog ('Zeit. für physiolog. Chemie,' vol. 41, p. 416 (1904).), in which the attempt is made to apply Nernst's diffusion hypothesis to all enzyme actions. Without further investigation it is impossible to pronounce a definite opinion on the matter, and Herzog's re-calculations of Henri's results scarcely serve to prove the truth of his premises. Having regard to the results of Henri ('Lois Générales de l'Action des Diastases,' Paris, 1903), A. J. Brown ('Journ. Chem. Soc.,' vol. 81, p. 393, 1902), and others, and to the temperature coefficient of enzyme actions as determined by O'Sullivan and Tompson ('Journ. Chem. Soc.,' vol. 59, Part I, p. 834 (1890)), and Müller-Thurgan ('Thiels Wirtschaftliche Jahrbuch,' 1885, p. 795), it seems not unlikely that the relative parts played by diffusion and by the actual chemical change in determining the reaction velocity depend upon the conditions of experiment.

§ Compare Kastle and Loevenhart, 'Am. Ch. J.,' vol. 29, pp. 397 and 563 (1903).

apparently no strongly oxidising intermediate peroxides are formed,* nor is "active" oxygen evolved. It was also found that the action has exactly the same initial velocity whether the solutions be carefully freed from dissolved oxygen by a current of hydrogen, or whether, as usual, a little of the former gas be present.

The Action of Poisons.—The question as to the mechanism of the action of "poisons" on the hæmase catalysis of hydrogen peroxide may now be considered. If the enzyme exists as a colloid in solution several explanations† seem possible:—

(*a*) Part of the enzyme may be rendered inactive by forming a chemical compound with the poison.

(*b*) Part of the surface of the particles may become covered with a thin layer of the poison or one of its decomposition products, thus preventing further action on the peroxide.

(*c*) The relation of the particles to the surrounding medium may be altered in various ways (change of surface tension, alteration of relative difference of potential, &c.) through addition of a poison.

Doubtless other explanations may be suggested. It is also exceedingly probable that all poisons do not act in the same way.

In this connection it may be noted that, according to Kastle and Loevenhart, the retarding effect of poisons on the catalysis of H_2O_2 by metals and other inorganic catalysors is due in most cases to formation of an insoluble film (compound between poison and catalysor) on the surface of the catalysor.

The action of acids and alkalies on the hæmase catalysis is of particular interest, since it has been shown, more especially by Hardy,‡ that minute traces of these bodies affect profoundly the relation between the colloidal particles and the surrounding medium. According to Linder and Picton,§ Hardy,‖ and others, the effect of acids in precipitating colloids is proportional to their electrical conductivity, *i.e.*, to the hydrogen ion concentration, but this is probably not connected with the similar effect on the hæmase catalysis because the former action is irreversible, whereas I have shown that the latter is reversible. It cannot be said that any very satisfactory explanation of the effect of acids, alkalies, and other electrolytes on colloids has yet been given.‖

With regard to the reaction under consideration I am inclined, in most cases, to favour a chemical explanation of the toxic effect. Acids,

* Or, if formed, their velocity of decomposition, either of themselves or in contact with hydrogen peroxide, is much greater than the reaction velocity between them and such a reducing substance as arsenious oxide.

† Compare Bredig, *loc. cit.*, p. 86.

‡ 'Roy. Soc. Proc.,' vol. 66, p. 110 (1899—1900).

§ Linder and Picton, 'Journ. Chem. Soc.,' vol. 67, p. 66 (1895).

‖ *Vide* Hardy, *loc. cit.*, p. 124; Bredig, *loc. cit.*, pp. 9—22; Freundlich, 'Zeit. phys. Chemie,' vol. 44, p. 129 (1903).

for example, may form with the enzyme compounds which are inactive towards hydrogen peroxide. If the affinity between hæmase and the acid is small, in other words, if the enzyme acts as a weak base, the amount combined, and consequently the retardation will be proportional to the strength of the acid. We have already seen that hæmase and most other enzymes are rendered inactive by small quantities of alkali, and that they regain their activity on the solution being neutralised. These facts seem to lend some support to a suggestion I have already made on a former occasion,* that at least some enzymes belong to the class of amphoteric substances which are, under ordinary circumstances, neutral, but in the presence of bases develop acid properties and can combine with acids to form salts. Within the last few years it has been shown that many albuminous substances are amphoteric.

The slight retardation caused by most neutral alkali salts is due, in all probability, to increased viscosity of the solution. This cannot apply, however, to the considerable toxic effect of the chlorine and bromine ions and, since a similar retardation of the platinum catalysis has been observed, it may be due to some action between the hydrogen peroxide and halogen salts. Kastle and Loevenhart† regard the retardation of the platinum catalysis as due to the formation of surface films of the insoluble platinous halides.

The retarding influence exerted on the action by oxidising agents is very remarkable. It is not due to oxidation of the enzyme in most cases, as is clear from the fact that the constants do not decrease during the action in presence of potassium perchlorate and nitric acid. On the other hand, potassium chlorate in exceedingly dilute solution seems to oxidise the enzyme, and since the neutral salt itself has very weak oxidising properties, its activity in this case must be due to the presence of the peroxide. That it is due to chloric acid set free by the hydrogen peroxide, acting as an acid, is scarcely likely, since the acidic properties of the latter are very weak. In this connection it is interesting to note that potassium persulphate has very little effect on the action, whereas persulphuric acid has a high oxidation potential.

The great retardation caused by some reducing agents, such as sulphuretted hydrogen and hydroxylamine, is also rather difficult to account for. Bredig‡ suggests that the poisonous effect of the former on the platinum catalysis is due to the deposition of sulphur on the surface of the platinum, whereas Kastle and Loevenhart regard it as being due to formation of a surface film of platinum sulphide. Neither of these explanations seems to apply to the effect on the hæmase catalysis, since I have observed that the constants increase considerably during

* *Loc. cit.*, p. 301.
† *Loc. cit.*
‡ *Loc. cit.*, p. 87.

the action (p. 209). It seems much more probable that the H_2S renders the enzyme inactive, perhaps by forming a loose compound with it, and that the gradual increase of the constants is due to recovery of the enzyme owing to oxidation of the poison. The toxic action of mercuric salts, and of hydrocyanic acid, seems most simply explicable on the theory of formation of loose compounds between them and the enzyme.

Summary of Results.

1. Quantitative measurements have been made of the effect of various substances on the rate of decomposition of hydrogen peroxide by hæmase, an enzyme obtained from blood.

2. The probable mechanism of the catalysis in question has been discussed, and reasons have been given for supposing that what is actually measured is the rate of diffusion of hydrogen peroxide to the enzyme.

3. The ways in which "poisons" may exert their effect on the action have been discussed, and reasons have been given for preferring, in some cases, a chemical explanation of the observed facts.

4. From the results obtained with acids and alkalis, as well as from the results of other observers, it has been concluded that at least some enzymes are amphoteric substances, *i.e.*, substances which are neutral in aqueous solution but can combine both with acids and with bases to form salts.

I have much pleasure in thanking Dr. Charles A. Kohn for the facilities he has kindly allowed me for carrying on my work.